Abstract No. schu162

High Resolution Data Collection for Three Crystal Forms of an Anti-ssDNA Antibody Fragment (Fab)

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Beamline(s): X8C

Introduction: Anti-DNA antibodies have been implicated in autoimmune diseases such as systemic lupus erythematosus (SLE), yet very little structural information is available for antibody/DNA complexes. Our lab has previously determined the crystal structures of an anti-ssDNA Fab bound to dT_5 and dT_3 (oligothymidine). The current focus involves the determination of the mechanism of ssDNA binding. For this study, three crystals forms of this Fab were grown in the absence of DNA ligands, and synchrotron radiation was utilized to obtain high resolution data.

Results: The crystallographic parameters for three unliganded crystal forms are given in Table 1. These data were obtained at Brookhaven National Light Source on beamline X8C. The trigonal crystal form came from a perfect merohedral twinned crystal. The crystal appeared to be P622, but crystal packing considerations showed significant overlapping solutions. Of the three crystal forms, the orthorhombic form diffracts to the highest resolution (1.75 Å). This is seen in Figure 1. These high resolution data have given insight into induced fit binding and cross-reactivity.

For each of the three crystal forms, 100^{o} of data were recorded with a 0.5^{o} oscillation angle collected on a Quantum 4 CCD detector. The exposure time ranged from 3-4.5 minutes per degree of oscillation depending on the crystal form. For all three unliganded crystal forms, two molecules are present in the asymmetric unit. The phases were determined using molecular replacement.

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References: S.P.Prewitt, A.A. Komissarov, S.L. Deutscher, J.J. and Tanner, "Crystallization and Molecular Replacement Studies of a Recombinant Antigen-Binding Fragment Complexed with Single-stranded DNA," Acta Cryst. **D56**, 1007-1011, 2000.

Table 1. Crystallographic parameters from three unliganded crystal forms.

Space Group	P321	P2(1)2(1)2	†P6(5)
Unit Cell	a=179.2 Å c=91.9 Å	a=107.7 Å b=156.7 Å c=61.0 Å	a=202.8 Å c=44.8 Å
Number of crystals	1	1	1
Resolution (outer shell)	50-2.50 Å (2.59–2.50 Å)	50-1.75 Å (1.81-1.75 Å)	50-2.30 Å (2.38-2.30 Å)
No. of observations	296346	338206	233009
No. of unique reflections	51454	98022	46702
Completeness (%)	87.0 (81.4)	93.5 (94.1)	99.8 (99.9)
Mean I/□	13.3 (2.9)	21.7 (2.1)	16.3 (3.6)
R_{sym}	0.090 (0.511)	0.044 (0.487)	0.085 (0.455)
No. of protein atoms	6323	6533	5383
No. of water molecules	48	784	0
No. of hepes molecules	0	5	0
No. of glycerol molecules	0	20	0
No. of PEG molecules	0	6	0
No. of sulfate ions	3	7	0
R_{cryst}	0.192 (0.258)	0.198 (0.300)	0.328 (0.410)
R_{free}	0.218 (0.292)	0.230 (0.317)	0.365 (0.405)
RMS deviations			
Bond lengths	0.010 Å	0.010 Å	0.009 Å
Bond angles	1.6^{o}	1.7^{o}	1.5^{o}
Dihedral angles	28	28	28
Improper dihedrals	1	1	1
Average B-factors (Å^2)			
Protein	33	26	43
Solvent	24	44	
Real space CC			
Protein	0.93 (0.03)	0.94 (0.04)	0.91 (0.06)
Solvent	0.95 (0.03)	0.87 (0.06)	

† Currently in structural refinement stages.

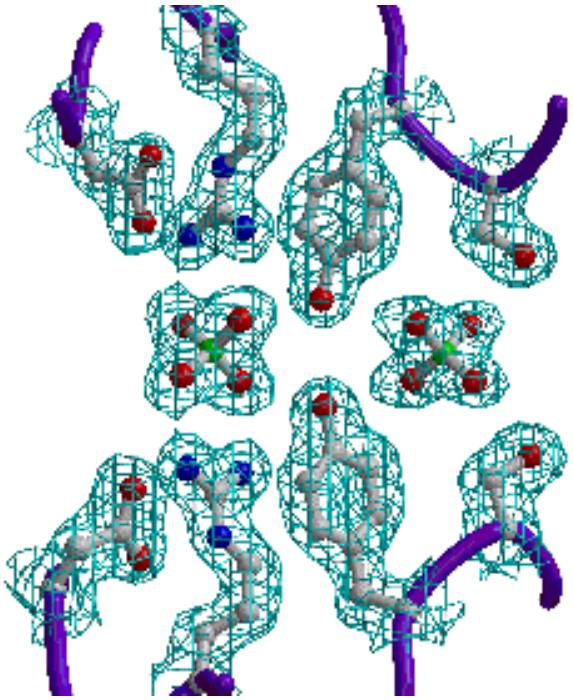

Figure 1. Special positions of sulfate ions in orthorhombic structure.